IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Gwynne Attarian, et al.

Application No.: 09/747,385

Filed: December 22, 2000

For: FUSOBACTERIUM NUCLEIC ACIDS, PLASMIDS AND VECTORS

Customer No.: 20350

Confirmation No.

Examiner: Davis, Katharine F.

Technology Center/Art Unit: 1636

STATEMENT UNDER 37 C.F.R.§1.804

Post-it® Fax Note 7671	Date 4/6	# of pages ▶ 2
To Elisabeth Sampson	From Susan K. Haake	
Co./Dept. TT+C	Co. UCLA	
Phone # 415/576-0200	Phone # 310/794-7163	
Fax # 415/576-0300	Fax # 310/206-3282	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Susan A. Kinder-Haake, am a named inventor on the above-referenced patent application and have control over the conditions of the deposit described herein.

I hereby confirm that the following biological deposits:

ATCC PTA-5816 (cell line comprising pFN2), and

ATCC PTA-5815 (cell line comprising pFN3)

referred to in the above referenced application, were deposited on February 13, 2004 with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110, where the deposits were granted the accession numbers PTA-5815 and PTA 5816. The deposit was made pursuant to the provisions of the Budapest Treaty and the viability of the deposit was tested on February 20, 2004. A copy of the Deposit Receipt and Viability Statement is attached hereto as Appendix A.

I also confirm that, subject to paragraph (b) of 37 C.F.R.§1.804, the deposited material is the same as that specifically identified in the above referenced patent application and was in Applicants' possession at the time the application was filed.

Respectfully submitted,

Susan A. Kinder Haake

Susan A. Kinder Haake D.M.D. Ph.D.

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 415-576-0200
Fax: 415-576-0300
Attachments
ERS:ers
60164931 v1

ATCC

10801 University Blvd • Manassas, VA 20110-2209 • Telephone: 703-365-2700 • FAX: 703-365-2745

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.

To: (Name and Address of Depositor or Attorney)

UCLA School of Dentistry
Section of Periodontics
Attn: Susan Kinder Haake, D.M.D., Ph.D.
10833 Le Conte Avenue
Los Angeles, CA 90095-1668

Deposited on Behalf of: The Regents of the University of California

Identification Reference by Depositor:	**Patent Deposit Designation**
Fusobacterium nucleatum subspecies polymorphum ATCC 10953	PTA-5815
Fusobacterium nucleatum 10113	PTA-5816

The deposits were accompanied by: __ a scientific description _ a proposed taxonomic description indicated above. The deposits were received February 13, 2004 by this International Depository Authority and have been accepted.

AT YOUR REQUEST: X We will inform you of requests for the strains for 30 years.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested February 20, 2004. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

Marie Harris

Marie Harris, Patent Specialist, ATCC Patent Depository

Date: March 1, 2004

cc: Elizabeth R. Sampson
Ref: Attorney Docket No: 02307E-099810US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No. 9499
Gwynne Attarian, et al.	Examiner: Lambertson, David A.
Application No.: 09/747,385	Technology Center/Art Unit: 1636
Filed: December 22, 2000	Declaration of Susan A. Kinder Haake Under 37 C.F.R. §1.132
For: FUSOBACTERIUM NUCLEIC ACIDS, PLASMIDS AND VECTORS	
Customer No.: 20350	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Susan A. Kinder Haake, being duly warned that willful false statements and the like are punishable under 18 U.S.C. 1001, by fine or imprisonment or both, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true.

2. I am a co-inventor of the invention disclosed and claimed in the above identified patent application. I have read and am familiar with the contents of the subject patent application.

3. I hold a Ph.D. (1993) in Microbiology from University of Texas, San Antonio, a D.M.D. (1979) from Tufts University, a Certificate in Periodontology (1985) and a M.Dent.Sc. (1985) from the University of Connecticut, and a Bachelor of Arts (1976) from Hamilton-Kirkland College. I am an Associate Professor of Dentistry, Sections of

Periodontology and Oral Biology and Medicine, and a member of the Dental Research Institute at the University of California Los Angeles.

4. My field of expertise is in molecular microbiology, including the characterization and use of bacterial plasmids in developing genetic transfer systems.

5. I believe that one skilled in the art of molecular biology would regard proteins with 90% sequence identity to SEQ ID NO:1 (repA) as functionally equivalent to the repA protein of the invention. Furthermore, I believe an individual skilled in the art would be able to identify such repA proteins given the teachings of the present specification in combination with the literature available at the time the present application was filed. Indeed, based on current knowledge of plasmid replicons, 90% is probably a very conservative estimate of the amount of sequence identity needed to assure functionality (*i.e.*, function would probably be conserved with even greater alteration of the amino acids).

6. Related Replication initiator proteins from theta-replicating plasmids of the iteron-containing class may exhibit more than 75% amino acid sequence divergence.

The RepA protein (SEQ ID NO:1) and iteron sequences (as exemplified by SEQ ID NO:3) of the invention comprise *Fusobacterium* plasmids that are members of a group of plasmids known as "theta-replicating plasmids of the iteron-containing class". The conclusion that pFN1 is a member of this class of plasmids is based on DNA sequence analysis of the replicon structure (*i.e.*, presence of iterons) and homology of the Rep protein with those of other theta-replicating plasmids. The features common to this class of plasmids are discussed in the accompanying del Solar review paper (Exhibit A).

The del Solar paper (Exhibit A) presents a comparative amino acid sequence analysis of the replication initiator proteins from theta-replicating plasmids of the iteron-containing class. This analysis, while not addressed specifically to the pFN1 replicon, is nonetheless applicable to the pFN1 replicon as the Rep proteins analyzed in Exhibit A are all functional components of theta-replicating plasmids with origin structures comparable to that of the fusobacterial plasmid pFN1.

The analysis presented in Exhibit A shows that replication initiator proteins such as RepA, typically exhibit much greater than 10% sequence divergence *i.e.* considerably less than 90% sequence identity. For example, comparison of the two most divergent members of the family of related replication initiator proteins, pPS10 and R6K, shown in Figure 2, on page 439 of Exhibit A, reveals that related replication initiator proteins from theta-replicating plasmids of the iteron-containing class may exhibit more than 76% amino acid sequence divergence *i.e.* less than 24% sequence identity. This percentage is quite striking, particularly if one considers that this calculated sequence identity between pPS10 and RK6 Rep proteins is based only on amino acids that are in *aligned* portions of the two proteins (in Figure 2, there are 223 aligned residues, of which 53 are identical). Unaligned portions of the two proteins *i.e.*, the additional segments of amino acids of the R6K protein lacking a corresponding amino acid in the pPS10 protein, are *not* taken into account in this calculation. Thus, in essence this means that the 24% identity figure *overstates* the identity.

To underscore the sequence variation possible among different, related replication initiation proteins, I calculated the percent sequence identity between the full length replication initiator proteins of plasmids pPS10 and RK6 using the sequences cited in the legend to Figure 2 panel A, in Exhibit A. I did a ClustalW alignment of the entire protein predicted amino acid sequence. For the full length amino acid sequences, a comparison of pPS10 versus R6K reveals that these related replication initiator proteins are 17.14% identical, 20% different but strongly similar, 7.62% weakly similar, and 55.24% different. Thus, in simple terms of identity versus non-identity, the two related replication initiator proteins are 17.14% identical and 82.86% non-identical.

7. In a family of related lactococcal plasmids, replication initiator proteins with 60 to 80% amino acid sequence identity are able to complement one another's function.

Another demonstration that related replication initiator proteins from theta-replicating plasmids of the iteron-containing class exhibit wide sequence divergence while retaining similar functionality is provided by the accompanying Seegers *et al.* research paper

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(Exhibit B). Seegers *et al.* show that in a family of related lactococcal plasmids, replication initiator proteins with only 60 to 80% amino acid sequence identity are able to complement one another's function. Thus, the proteins are functionally equivalent. Again, the relevance to the case under consideration is that these lactococcal plasmids are all theta-replicating plasmids with origin structures comparable to that of the fusobacterial plasmid pFN1.

8. Based on the data presented above, I believe that in fact much more than 10% of the amino acids in the repA sequence could be altered and the function of the repA replication protein would still be preserved. Furthermore, I believe that one of skill in the art would be able identify such functional repA proteins, without undue experimentation, based on the teachings of the specification in combination with the knowledge in the art at the time the application was filed.

The Declarant has nothing further to say.

Dated: 3/4/04 By: Susan A. Kinder Haake

Susan A. Kinder Haake D.M.D. Ph.D.

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 415-576-0200
Fax: 415-576-0300
Attachments
ERS:ers
60157104 v1